UNIVERSITY OF PENNSYLVANIA

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IN BACTERIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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DÖDERLEIN'S BACILLUS: LACTOBACILLUS ACIDOPHILUS

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In a paper entitled "Concerning the Vaginal Secretions and Vaginal Bacteria," read before the 4th Congress of the German Gynecological Society at Bonn in May, 1891, Albert Döderlein [1] described the cultural and physiological characters of the organism which has since borne his name. From Döderlein's description and from those of subsequent workers we may, for convenience, consider this organism morphologically as a gram-positive, nonspore-forming rod, sometimes quite long and slender, with square or very slightly tapering ends, occurring singly or in chains. Although written as a bacteriological study, the author drew certain conclusions as to the relation of vaginal secretions to puerperal fever. This phase of the study immediately created a widespread interest which gradually developed into one of the great scientific debates in modern surgery. In the controversy as to whether the germs of childbirth fever were to be found in the vaginas of sepsis cases or whether they were introduced by obstetrical examinations—a quite natural bone of contention at the time of the "antiseptic" versus "aseptic" debate—the bacteriological aspects of Döderlein's work received only minor consideration. However, a number of studies of the relative prevalence and cultivation of this organism have been contributed to the literature. Of these the articles by Kruse,[2] Basten,[3] Lowi,[4] Joetten [5] and Solomon [6] are of especial interest. A recent article by Lash and Kaplan [7] fails to take into account the suggestions of Joetten as to nomenclature and offers a specific name Lactobacillus vaginae (n. sp.) to a member of this group. This does not seem justified in the light of the present work.

Owing probably to the initial impetus of Döderlein's assertions and the resulting conflict of opinion, practically all of the studies of this

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1. Verhandl. d. deutsch. Gesellsch. f. Gynäkol., 1892, p. 35.
2. Allgemeine Mikrobiologie, 1910, chapt. 6, p. 97.
3. Ztschr. f. Hyg. u. Infektionskr., 1914, 77, p. 282.
4. Wien. klin. Wchnschr., 1920, 33, p. 730.
5. Archiv. f. Hyg., 1922, 91, p. 143.
6. Ztschr. f. Geburtsh. u. Gynäkol., 1922-3, 85, p. 554.
7. J. Infect. Dis., 1926, 38, p. 333.

bacterium have been on pregnant women. The present study had its inception in an entirely different field of inquiry. In an investigation of the problem of the diagnosis of gonorrhea in women, the author was struck by the fact that where bacteriological findings showed the gonococci in direct vaginal or cervical smears, Döderlein's bacillus was always absent. This observation gave rise to a number of theoretical questions which the present work attempts to answer.

Inasmuch as the literature from 1892 to 1900 abounds in conflicting findings of the bacterial flora of pregnant women it would seem that if there were such an organism as a vaginal bacillus, using the term in the same sense as we use the term the colon bacillus, and which seems to be Döderlein's idea, then the proper field for study would be in children below the age of puberty. For ease in handling the cases and in order to work with a definite age group, children between the ages of 6 and 12 were used exclusively in this study.

The technic used for obtaining smears and cultures was somewhat similar to that described by Harris and Brown [8] except that a modification of the Menge tube was employed. It did not seem that the disadvantages of the tube pointed out by Fricke [9] applied to the type of case under consideration. The swab-tube consists of a small, tightly wound applicator with the cotton end inserted midway into a straight glass tube very slightly drawn out at one end. Both ends of the tube are plugged with cotton and the apparatus inserted into a testtube which in turn is plugged with cotton. The entire outfit is then sterilized.

The following instructions were drawn up and were used as the standard procedure in collecting all vaginal specimens for culture and direct smear.

Technic for Culture and Smear of Vaginal Secretion.—Materials: Tray, green soap, sponges, sterile water, mercuric chloride 1 : 2,000, sterile towels, package of sterile sponges, sterile drapings, and a bag for discarded sponges; and sterile swabs, petri dish plates of whey agar, clean microscopic slides.

Procedure: The nurse will provide all materials except swabs, petri plates and slides, and will screen and drape the patient. Children between 6 and 12 years (both inclusive) will be examined. The operator will scrub his hands thoroughly with green soap and water, paying particular attention to finger nails. Immerse hands in mercuric chloride 1 : 2,000, dry hands on a sterile towel, powder and put on sterile gloves. Sterile drapings will be laid over the other drapings. The labia are separated with the thumb and first finger of the left hand, until the procedure is over. The vaginal introitus is washed with green soap and water (suds) using four sponges with a downward stroke and not using the same

8. Am. J. Obst. & Gynecol., 1926, 11, p. 4.
9. Cited in Williams: Obstetrics, 1924, p. 997.

sponge twice. Follow with sterile water using three sterile sponges, then mercuric chloride using 6 sterile sponges, and then again with sterile water using 4 sterile sponges, always washing from above downward and discarding the sponge at once.

A sterile swab is inserted into the vagina and the vagina is thoroughly swabbed with a circulatory movement. The swab is then removed and immediately spread over two whey agar plates, and then over a slide for the smear. The inoculated plates are placed immediately into a container held in a water bath at 40 C., and transferred as soon as possible to the laboratory where one set was placed directly in the incubator (aerobically) and the other into a vaccum jar and, after drawing a vacuum, into the 37 C. incubator. In the early part of the work pyrogallic acid and sodium hydroxid were placed in the vacuum jar immediately before drawing the vacuum but later this procedure was abandoned. Both sets of plates were incubated for 48 hours. Fishings were made and the organisms isolated were classified.

In all, 107 smears and plate cultures were made from normal vaginas. Subcultures were made from all different colonies on both aerobic and anaerobic plates in the first 50 cases, after that only the organisms resembling Döderlein's bacillus were subcultured. This procedure accounts for the apparent discrepancy between the microscopic and cultural findings.

In most of the cases from which it was isolated Döderlein's bacillus was found growing in the anaerobic plates only, in one case it was found in the aerobic plate only. In 12 cases it was apparent in direct smear but could not be isolated culturally. Although it might appear from the table that there were a large number of organisms present in smears and cultures this was not the case. In 11 cases no organisms were found either in direct smear or culture, in 18 cases the plates were sterile. In practically every case only a few organisms of each kind listed were found. In general the findings agree most closely with those of Williams [10] except that the Döderlein bacillus does not appear in children as frequently as he found it in adults.

For determining the reaction of the vagina a small glass distillation bulb equipped with a rubber bulb and containing about 5 cc. of sterile distilled water was used. The bulb was inserted into the vagina immediately after the withdrawal of the swab tube and the water forced into the vagina with some little pressure. The rubber bulb was then allowed to expand withdrawing the water back into the glass bulb. The reaction of this washing was determined. In every case where Döderlein's bacillus was found the reaction approached P_H 6.0 (5.6 to 6.2). In cases where Döderlein's bacillus was not found the reaction was

10. Am. J. Obst., 1898, 38, pp. 449 and 807.

around P_H 6.8 (6.7 to 6.9). In two cases only, both of vulvovaginitis and not recorded in table 1 (normal vaginas), was the reaction above P_H 7.0 (7.2 to 7.3).

Various mediums were used in the early part of the work for subculturing Döderlein's bacillus. Huntoon's hormone agar, 3% glycerol agar, 0.5% and 1.0% lactose agar, blood agar, whey agar, whey agar fortified with 1% galactose, trypsin digest agar and liquid media of the same compositions without the agar were all tried without any showing a striking advantage. For the routine carrying of the cultures milk and 1% galactose whey were finally decided upon. Both of these media seem to support the growth of the organism equally well and they have the advantage over solid media in not drying readily, an environmental factor to which the organism is very susceptible.

TABLE 1.—MICROSCOPIC AND CULTURAL FINDINGS FOR 107 SPECIMENS FROM NORMAL VAGINAS

	Smears	Cultures
Döderlein's bacillus	20	8*
Staphylococci	96	49
Diphtheroids	78	30
B. coli (group)	91	48
Streptococci	40	20
Sarcina	10	5

* In a preliminary tabulation of the cultures isolated all were referred by notation to the individual cases examined. As such a table would be too cumbersome for the purpose of this paper, the Döderlein strains are simply called D1, D2, D3, etc. in the time order of their isolation.

For the first few transplants the organism grew much better under anaerobic conditions. Now, after carrying them for over a year, they grow very well under slightly lowered oxygen tension, and some strains grow well aerobically. The reaction of the medium within rather large limits does not have much influence on the growth of Döderlein's organism. In taking the same lot of whey, and dividing it into several lots and adjusting the reaction between P_H 4.8 and P_H 7.6 with lactic acid we obtained a good growth of the bacillus in all tubes. Tubes planted with B. coli and Staphylococcus aureus failed to show growth below P_H 5.3. The alkaline point P_H 7.6 was not exceeded in attempting to show the organism's tolerance for alkalinity as this point is well above that met with in any vaginal reaction.

The power of acid production and the rate of acid production of the organism varies slightly with different strains. Table 2 shows the average end points of acid production in various lots of whey, milk and trysin digest, bouillon, all fortified with the same amount (1%) of lactose.

There seems to be some relationship between the morphology of the cell and the colony formation. Both of these characteristics show a decided tendency to change, even when subcultured into the same medium. Upon original isolation three different morphological types were recorded. Three of our strains were slender rods 0.5μ in diameter and from 3 to 6μ in length, occasionally appearing in short chains of three to six members. Three strains were of about the same shape and size as those above but they showed a rather noticeable palisade formation. Two of our strains were heavy rods, 0.7 to 1μ in diameter and 10 to 15μ in length. They formed long filaments. All

TABLE 2.—ACID PRODUCTION BY DÖDERLEIN'S BACILLUS (D), LACTOBACILLUS ACIDOPHILUS (A) AND BULGARICUS (B)

Medium	Organisms	P_H
Milk	D 1, 2, 4, and 5; A 1 and 2; B 1 and 2	3.5
	D 7	3.6
	D 6	3.7
	D 3	3.9
	D 8	5.5
Whey	D 1, 2, and 6	3.5
	D 3; A 1 and 2	3.8
	D 4, 5, and 7; B 1	3.9
	B 2	4.3
	D 8	5.4
Trypsin	D 7	3.8
	D 1, 2, 3, 4, and 5	3.9
	A 1	4.0
	B 2	4.1
	D 8; B 1	5.0

D 1, D 2, etc. indicate the eight different strains of Döderlein's bacillus isolated, B 1 and B 2 are two strains of Lactobacillus bulgaricus, and A 1 and A 2 are two strains of Lactobacillus acidophilus used as controls in this experiment.

strains were uniformly gram-positive with square ends. All were non-motile and nonspore-forming.

The colonies on the first plates also divided themselves into three groups. The first was smooth, circular, convex, entire, amorphous and translucent. The second was rough, rhizoid, contoured, flat, and lacerate, resembling a typical Bulgaricus colony but not as irregular. The third type was something in between the two. Six of our colonies were of the first group and one of each the latter two. With the exception of one organism, D2, every one of these strains has changed during the past eighteen months both in cell morphology and in colony formation. An exhaustive study of these changes, with camera lucida drawings and photomicrographs of colonies and colony impressions has been made and is still in progress. Space, however, forbids a further record of our findings here.

As to temperature requirements all strains grow best at 37.5 C. or slightly higher. Below 35 C. there is practically no growth, and none of the strains grow at all at 20 C.

All of our strains are facultative anaerobes. All grow better in a partial (10%) carbon dioxide atmosphere, or in an atmosphere in which the oxygen is further diluted with nitrogen gas. When air instead of CO_2 or N_2 was bubbled through a liquid substratum no better growths were obtained, indicating that an excess of moisture could not account for the increased growth in the presence of these gases.

Good growth was obtained with every strain of Doderlein's bacillus when the surface tension was reduced to 40 dynes, using sodium ricinoleate as a depressant. Controls with Lactobacillus acidophilus grew in the same depressed medium while controls with Lactobacillus bulgaricus did not. None of our strains produced any growth on potato, none produced indol from a tryptophane broth, and all reduced nitrates to nitrites.

In sugar fermentations different strains of Döderlein's bacillus acted differently. The medium used was Huntoon's hormone broth fermented for 48 hours with B. coli. To this sugar-free broth was added 1% of the different carbohydrates. Table 2 shows our results, with cultures of Lactobacillus acidophilus and Lactobacillus bulgaricus as controls. The strains D6, D8, do not always ferment maltose equally well, at times producing only a slight acidity. D1 at times does not ferment sucrose, D4 ferments sucrose usually but not always. D6 is variable on mannitol. D1, D3, D4, sometimes ferment levulose very slightly. None of the strains produces gas in any of the carbohydrate media. In the ability to ferment maltose all of our Döderlein strains correspond to the classical description of Lactobacillus acidophilus. Two of our strains, however, while producing acid from this sugar only form a slight amount at times. The other irregularities noted seem to signify that Döderlein's bacillus forms a heterogeneous group in fermenting powers, not falling into Rahe's groups with any constancy.

In milk some differences have occurred in the type of coagulum but these have not been constant with any strains and may depend on the rate of acid production formed, that is, in overcoming the lag. As a rule the coagulum was soft.

Strains of Döderlein's bacillus were inoculated into rabbits for cross agglutination studies. Strains of Lactobacillus acidophilus and Lactobacillus bulgaricus were used as controls. Table 4 summarizes the results obtained. The serums agglutinated their homologous antigens in every case at a dilution of 1 : 5000 or over. Bulgaricus serum did

not agglutinate any of the other antigens and none of the other serums agglutinated L. bulgaricus antigen. The group agglutinins ran to approximately 1 : 160. This point was taken as the group agglutination point. The plus (+) and zero (0) signs in the table indicate whether or not agglutination took place above that dilution. It will be noted that there is no grouping of strains possible by the agglutination test. This fact was borne out by agglutinin absorption tests.

Every strain of our Döderlein's bacillus has been reisolated from feces of human beings after feeding. Organisms for this purpose were fed in a 48-hour incubated milk (1 pint) culture. Approximately two ounces of lactose were added to each bottle of milk just before using.

TABLE 3.—FERMENTATION REACTION WITH DÖDERLEIN'S BACILLUS, LACTOBACILLUS ACIDOPHILUS AND BULGARICUS

Organism	Monosaccharides						Disaccharides			Trisaccharides	Polysaccharides		Alcohols	
	Dextrose	Levulose	Galactose	Xylose	Arabinose	Mannose	Maltose	Lactose	Dulcrose	Raffinose	Inulin	Salicin (glucoside) Dextrin	Mannitol	Dulcitol
D 1	+	0	+	0	0	+	+	+	+	+	0		+	0
D 2	+	+	+	0	0	+	+	+	0	0	0		+	0
D 3	+	0	+	0	0	+	+	+	+	0	0		0	0
D 4	+	0	+	0	0	+	+	+	+	0	0		0	0
D 5	+	+	+	0	0	+	+	+	0	0	0		0	0
D 6	+	+	+	0	+	+	+	+	0	0	0		+	+
D 7	+	+	+	0	0	+	+	+	0	0	0		0	0
D 8	+	+	+	0	0	+	+	+	+	0	0		0	0
A 1	+	+	+	0	0	+	+	+	+	0	0		+	0
A 2	+	+	+	0	0	0	+	+	0	0	0		0	0
B 1	+	+	+	0	0	+	0	+	+	+	0		+	0
B 2	+	+	+	0	0	+	0	+	+	0	0		+	0

From the morphologic, cultural and physiologic characteristics of Döderlein's bacillus there is no doubt but that we are dealing with Lactobacillus acidophilus. However, we are convinced that the name Lactobacillus acidophilus either characterizes a group of related species or that, if it is a single biologic species, this species undergoes transformations, maybe "dissociations" which are in truth remarkable. In every mark which recent workers have distinguished Lactobacillus acidophilus from Lactobacillus bulgaricus this organism agrees with the former. Until, therefore, we know more about Lactobacillus acidophilus no new specific names for the vaginal bacillus are justified.

Assuming then that Döderlein's bacillus is Lactobacillus acidophilus, the question naturally arises, "How does Lactobacillus acidophilus get into the human vagina?" Theoretically the answer seems to lie with

its bacterial associates in the vagina. B. coli, diphtheroids and staphylococci are usually present in the vagina. Staphylococci are present on all surfaces of the body but B. coli and the diphtheroids are normal inhabitants of the extremely close body orifice—the rectum. By pure deduction therefore it would seem that Lactobacillus acidophilus gets into the vagina from the intestinal tract.

Two experiments were carried out to sustain this thesis. One was to examine the fecal contents of individuals showing the organism in the vagina, and the other to attempt to plant Lactobacillus acidophilus in the vagina by way of the digestive tract. Out of six cases examined in which this organism was found by smear to be in the vagina, the organism appeared in direct fecal smear in five. In one case the organism was found in the vagina but not in the feces. Two

TABLE 4.—CROSS AGGLUTINATIONS WITH SPECIFIC DÖDERLEIN'S BACILLUS ANTISERUMS AND ANTIGENS

Anti-serums	Antigens									
	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	A 1	B 1
D 1	+	+	0	+	+	0	+	+	+	0
D 2	+	+	0	+	+	0	+	+	0	0
O 3	0	0	+	0	+	0	+	+	+	0
D 4	+	+	0	+	+	+	0	0	0	0
D 5	+	+	+	+	+	0	0	0	+	0
D 6	0	0	0	+	0	+	0	0	+	0
D 7	+	+	+	0	0	0	+	+	+	0
D 8	+	+	+	0	0	0	+	+	+	0
A 1	+	0	+	0	+	+	+	+	+	0
B 1	0	0	0	0	0	0	0	0	0	+

children who did not show Lactobacillus acidophilus in their feces or vagina were fed the organism strain D1 in milk. In both of these cases the organism was reisolated from the feces and from the vagina. Acidophilus feeding was stopped and examinations continued. In one case the organism disappeared from the feces within two weeks and from the vagina within three weeks. In the other case the organism persisted in the feces and in the vagina for more than three months. It then disappeared from the feces but remained in the vagina for at least three months longer when the case was lost to us.

This brings up the question of what the organism lives on in the vagina and from what it produces its acid. There are two possibilities. First that unfermented fecal matter is mechanically transferred from the anus to the vagina in sufficient amount for it to maintain its existence, and second that it finds a nutrient material in the vagina. The first of these alternatives is hard to eliminate. Extracts of normal

feces contained fermentable substances. Meconium extracts did not. On the other hand, normal sterilized vaginal washings had material which was fermentable by all of our strains isolated and artificially purified mucus from the mouth was fermentable. Even when no acidophilus is found in the vagina the vaginal washings are slightly acid which seems to indicate that there is a secretion in this organ not wholly derived from the cervix as the secretions from the racemous glands in the cervix are alkaline. It would seem that the vaginal fluid is serous in character and contains a fermentable mucin.

A study of the intestinal contents of children from birth showed the change in flora follows that described by Tissier.[11] The meconium is sterile. On the second day gram-positive rods appear which prove to be B. bifidus. This organism continues dominant in breast fed children for from one to four weeks. Other organisms such as the enterococcus appear soon and in the case of bottle fed babies, Lactobacillus acidophilus. B. coli gradually appears in all cases but remains in the minority. As far as we could determine the changes in the early intestinal flora have no effect on the vaginal flora. In no case did we find Lactobacillus in vaginal smears from the thirteen infants whose intestinal flora we studied.

Having studied the morphologic, physiologic and cultural characters of Döderlein's bacillus and classified it as Lactobacillus acidophilus, having attempted to find out its relative incidence in the human vagina, its source and mode of entrance and on what it lives and produces acid in the vagina, we again returned to our original question, "What is the significance of this organism in gonorrheal infections?" The adult vagina is so exposed to external conditions it seemed that the problem could best be attacked with the study of vulvovaginitis in children. Unfortunately we have not had sufficient cases to form any decided opinions. The two cases which we have studied, however, seem highly significant.

Case 1.—A child of 8 years, suffering with acute vulvovaginitis, had come into the hospital about six months previously and had a gonococcic vaginitis at that time. This case was chronic and had not responded to treatment. At the time we first took over the case the condition had become acute with a marked discharge in which the gonococci were very numerous. All treatment was suspended for one week and then the vagina was flushed thoroughly with a culture of Lactobacillus acidophilus (strain Dl) daily. This was the only treatment given. For two weeks the reaction of the vagina before flushing with the culture remained alkaline (PH 7.3) although smears showed the absence of the gonococci in 48 hours. At the end of 96 hours after the first flushing pus cells disappeared. Treatment was stopped after three weeks with a vaginal reaction PH 6.6. This case has been under observation for more than a year and weekly vaginal smears have not shown any gonococci. Once or twice pus cells have been noted but no laboratory or clinical signs of the vulvovaginitis have reappeared.

11. Recherches sur la flore intestinale normale et pathologique dus nourrisons, 1900, p. 85.

Case 2.—A child, three years of age, brought into the hospital, showed gonococci in vaginal smear. Vaginal flushings were made with a culture of Lactobacillus acidophilus (strain D1). Forty-eight hours later no gonococci were present in smears and only a few pus cells. The latter disappeared completely in five days. The child was taken home on the tenth day and no subsequent record was obtainable.

From the results of only two cases no definite statements as to the efficacy of this treatment are warranted. In the light of the fact that Lactobacillus acidophilus was not found in any case of gonorrhea in the female, and that the acid produced by this organism is decidedly detrimental to the growth of the gonococcus, the results are significant.

Laboratory cultures of the gonococcus on blood whey agar are killed within 18 hours when a whey broth culture of Lactobacillus acidophilus is spread thinly over them.

CONCLUSIONS

Döderlein's vaginal bacillus is Lactobacillus acidophilus. This organism is present in less than 10% of normal vaginas of children. It gets into the vagina by exterior passage from the intestinal tract, and can be introduced into the vagina by feeding a culture by mouth. It lives and develops acid either from a secretion of the vagina or in conveyed fermentable intestinal material or both. The organism is not present in the vagina in cases of gonococcal vaginitis. The organism has an inhibiting effect on the growth of the gonococcus in vitro, and Lactobacillus acidophilus vaginal implantation may present a rational cure for gonococcal vulvovaginitis and other gonococcal infections.